The tissue cyst in this chinchilla was probably an incidental finding. The chinchilla probably became infected by ingesting food contaminated with feces of an infected hawk. The tissue cyst phase of *Frenkelia* generally does not cause clinical signs in animals (Dubey et al., 1989). Although the merogonic phase of *Frenkelia* spp. in naturally infected animals is unknown, merogony occurs in hepatocytes in experimentally infected animals, and can be pathogenic (Rommel and Krampitz, 1975; Gobel et al., 1978). *Frenkelia* meronts are structurally similar to those of *Sarcocystis* spp that develop in parenchymal cells, e.g., *Sarcocystis muris* with cat–mouse cycle. Acute hepatic sarcocystosis was reported in 2 chinchillas by Rakich et al. (1992), and has been seen in 3 other chinchillas in the United States (J. Dubey, unpublished observation). Whether acute hepatic sarcocystosis in chinchillas represents a phase of *F. microti* needs investigation. In this respect, of the 22 chinchillas necropsied over the past year at the Naval Medical Center, San Diego, 14 had gross hepatic lesions with an undetermined etiology.

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Immunohistochemical Confirmation of *Sarcocystis neurona* Infections in Raccoons, Mink, Cat, Skunk, and Pony

J. P. Dubey and A. N. Hamir*, Parasite Biology and Epidemiology Laboratory, Livestock and Poultry Sciences Institute, ARS, USDA, BARC-East, Beltsville, Maryland 20705; *National Animal Disease Center, ARS, USDA, 2300 Dayton Road, Ames, Iowa 50010

ABSTRACT: In the central nervous system of 2 raccoons, 1 cat, 1 pony, 2 mink, and 1 skunk, protozoa previously thought to be *Sarcocystis*-like reacted positively to *Sarcocystis neurona*-specific antibodies in an immunohistochemical test. In addition, *S. neurona* was identified in the brain of another skunk. These observations indicate that *S. neurona* is not confined to opossums and horses.

Sarcocystis neurona is an etiologic agent for equine protozoal myeloencephalitis (EPM) in horses, and EPM is the most common cause of neurologic disorders in horses in the Americas (Dubey et al., 1991; Hamir et al., 1992; MacKay, 1997). The life cycle of *S. neurona* is not fully known. The opossum (*Didelphis virginiana*) is a definite host, and horses are considered aberrant intermediate hosts. Horses are thought to become infected by ingesting *S. neurona* sporocysts excreted in the feces of opossums (Fenger et al., 1997; Dubey and Lindsay, 1998). How opossums become infected with *S. neurona* is not known because intermediate hosts harboring *S. neurona* sarcocysts are not known. Only schizonts and merozoites are found in tissues of horses, and these stages are confined to the brain and the spinal cord. Live *S. neurona* has been isolated only from the central nervous system (CNS) of horses and the intestines of opossums (Dubey et al., 1991; Dubey and Lindsay, 1998). Recently, *S. neurona*-like infections were found in sea lions from California and a sea otter from Oregon; the protozoa in the CNS of these animals reacted with *S. neurona* antibody (Lapointe et al., 1998; Rosonke et al., 1999). More recently, *S.*

TABLE I. Detection of *Sarcocystis neurona* by immunohistochemistry in brains of naturally infected animals in the United States.

Species	Location	Reference
Pony	Maryland	Dubey and Miller (1986)
Mink	Oregon	Dubey and Hedstrom (1993)
Cat	California	Dubey et al. (1994)
Skunk	Massachusetts	Dubey et al. (1996)
	Oregon	this study
Raccoon	Ohio	Dubey et al. (1990)
	New York	Stoffregen and Dubey (1991)

neurona was cultivated in vitro from the brain of a naturally infected sea otter (Lindsay et al., 2000). Previous to that, *S. neurona*-like infections were reported in raccoons (Dubey et al., 1990; Stoffregen and Dubey, 1991; Thulin et al., 1992), skunks (Dubey et al., 1996), mink (Dubey and Hedstrom, 1993), a cat (Dubey et al., 1994), a monkey (Klump et al., 1994), and a pony (Dubey and Miller, 1986).

Although antibodies to *S. neurona* have been reported in ponies and other equids, clinical EPM has been confirmed only in horses (Saville et al., 1997). The only case of EPM in a pony is that reported by Dubey and Miller (1986) before the discovery of *S. neurona*. Recently, a high-titer *S. neurona*-specific serum was produced in a rabbit using cultured merozoites (Dubey et al., 1999). Here, we report specificity of this serum and confirm *S. neurona* infections in various species of wild and domestic animals.

Tissues of 2 raccoons, 2 mink, a skunk, a cat, and a pony with *S. neurona*-like infections previously reported (Table I), tissues of a monkey (Klumpp et al., 1994), and tissues of an additional skunk from Corvallis, Oregon, with multifocal nonsuppurative encephalitis were used for immunohistochemical (IHC) demonstration of the parasites.

The *S. neurona* antibody was obtained from the serum of a rabbit injected with cultured merozoites of an *S. neurona* isolate from opossum no. 95 (Dubey et al., 1999). The positive control tissues were obtained from gamma-interferon knockout mice injected with *S. neurona* cultured merozoites and mice fed sporocysts from opossum feces (Dubey and Lindsay, 1998). The positive equine tissues were from the spinal cord of the horse that was used to describe the morphological characteristics of *S. neurona* (Dubey et al., 1991) and an EPM horse in which infection was verified by isolation of the SN6 isolate of *S. neurona* (Dubey et al., 1999). The negative control tissues used for the demonstration of specificity of the test serum were sarcocysts and second generation schizonts of *Sarcocystis cruzi* from cattle, schizonts from the liver of a horse infected with an undetermined species of *Sarcocystis* (Davis et al., 1999), tissues from a *Sarcocystis canis*-like infection in bears (Zeman et al., 1993; Garner et al., 1997), tissues from a chinchilla with hapatic sarcocystosis (Rakich et al., 1992), schizonts and sarcocysts of *Sarcocystis speeri* from mice (Dubey and Lindsay, 1999), tachyzoites and tissue cysts of *Neospora caninum* from mice, dogs, and cattle, *Neospora*-infected spinal cord from a naturally infected horse (Hamir et al., 1998), tachyzoites and tissue cysts of *Toxoplasma gondii* from cats and mice, and sarcocysts of *Sarcocystis kirkpatrickii* from raccoons (Snyder et al., 1990). For the IHC technique, the sections were deparaffinized, digested in 0.4% pepsin, and reacted with a 1:10,000 dilution of serum from the rabbit using the avidin–biotin peroxidase complex method (Dubey et al., 1999). The rabbit serum had a ≥1:16,000 titer in an indirect fluorescent antibody test using *S. neurona* merozoites from cell culture as antigen.

Organisms from all brains of animals in Table I (including 1 skunk from Oregon) reacted with the *S. neurona* antibody. Organisms in the spinal cord of the monkey previously considered to be *S. neurona* (Klumpp et al., 1994) did not react with *S. neurona* antibody in the present study. These findings suggest that another *S. neurona*-like organism might have been associated with encephalomyelitis in the monkey.

Tissues infected with other organisms did not react with the *S. neurona* antibody. Results of this study confirm that *S. neurona* infections occur in animals other than horses and opossums. The role of these animals in the life cycle of *S. neurona* remains to be determined.

Until recently, there was no immunocompetent animal model to study pathogenesis of *S. neurona*. Horses fed *S. neurona* sporocysts developed clinical signs and lesions consistent with EPM and had antibodies to *S. neurona*, but the parasite was not demonstrable in equine tissues (Fenger et al., 1997). Thus, Koch's postulates were not fulfilled. Although clinical EPM has been induced in immunodeficient mice (Marsh et al., 1997; Dubey and Lindsay, 1998), the small size of mice precludes evaluation of many clinical parameters. Findings of *S. neurona* in association with lesions in CNS tissues of nonequid animals indicates that these animals may also serve as natural aberrant intermediate hosts for *S. neurona*.

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Gyrodactylus anguillae (Monogenea: Gyrodactylidae) From Anguillid Eels (*Anguilla australis* and *Anguilla reinhardtii*) in Australia: A Native or an Exotic?

I. Ernst, A. Fletcher*, and C. Hayward†, Department of Microbiology and Parasitology, The University of Queensland, Brisbane, Queensland 4072, Australia; *Queensland Department of Primary Industries, P.O. Box 6014, Rockhampton Mail Centre, Rockhampton, Queensland 4702, Australia; and †Laboratory of Fish Diseases, Department of Aquatic Bioscience, Graduate School of Agriculture, University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113-8657, Japan

ABSTRACT: A species of *Gyrodactylus* collected from 2 species of anguillid eels (*Anguilla australis* Richardson, 1841 and *Anguilla reinhardtii* Steindachner, 1867) from Australia is identified as *Gyrodactylus anguillae* Ergens, 1960. The morphology of sclerites of *G. anguillae* specimens from Australia is in accordance with previous descriptions of specimens collected from *A. anguilla* (Linnaeus, 1758) from Europe and *A. anguilla* imported into Japan. *Gyrodactylus anguillae* was previously thought to be a parasite specific to *A. anguilla,* an eel that is native to freshwater catchments throughout Europe. Information on eel translocations and host and parasite biology is reviewed and it is hypothesized that *G. anguillae* is a naturally occurring parasite in Australia and not an introduction.

Despite increasing interest in the culture of anguillid eels in Australia, little attention has been given to their parasite fauna on this continent. Kennedy (1995) provides the only thorough investigation of the parasite fauna of a species of eel (*Anguilla reinhardtii* Steindachner, 1867) in Australia. Kennedy (1995) identified 2 species of Monogenea, namely *Pseudodactylogyrus anguillae* (Yin and Sproston, 1948) and *Pseudodactylogyrus bini* (Kikuchi, 1929), but not another well known parasite of eels, *Gyrodactylus anguillae* Ergens, 1960. We have found a species of *Gyrodactylus* on 2 species of anguillid eels from several localities in Australia. In the present paper, we provide a description of this species of *Gyrodactylus* and discuss whether it is an exotic or native parasite in Australia.

Infected *Anguilla australis* Richardson, 1841 and *A. reinhardtii* were collected from sites in Queensland, New South Wales, and Victoria. Specific localities are given below. Before being examined for parasites, eels collected from the Albert River in southeast Queensland were cultured for almost 1 yr at either the Queensland Department of Primary Industries (QDPI) Freshwater Fisheries and Aquaculture Centre (FFAC), Walkamin, north Queensland or the QDPI Bribie Island Aquaculture Research Centre (BIARC), southeast Queensland. Eels from other localities were dissected soon after capture. Fish were identified using Allen (1989). For the study of sclerites, parasites were mounted in ammonium picrate glycerin (Malmberg, 1970).